

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: www.elsevier.com/locate/apjtm

Document heading doi:

Cytokine profile in murine toxoplasmosis

Funda Dogruman–Al¹, Isil Fidan^{1*}, Bekir Celebi², Emine Yesilyurt¹, Berna Erdal¹, Cahit Babur², Semra Kustimur¹¹D1Gazi University Faculty of Medicine, Department of Microbiology and Clinical Microbiology, 06500, Besevler, Ankara, Turkey²Parasitology Laboratory of Communicable Diseases Research Department Refik Saydam National Hygiene Center, 06100, Sıhhiye, Ankara, Turkey

ARTICLE INFO

Article history:

Received 23 November 2010

Received in revised form 7 December 2010

Accepted 15 December 2010

Available online 20 January 2011

Keywords:

Murine toxoplasmosis

Cytokine

Pathogenesis

ABSTRACT

Objective: To investigate which cytokines are produced after acute infection of mice with *Toxoplasma gondii* (*T. Gondii*) RH strain. **Methods:** *Mus domesticus* domesticus mice in infected group were inoculated with with highly virulent *T. Gondii* RH strain by intraperitoneally. Serum samples were obtained from infected and non-infected mice for cytokine levels for ELISA assay. **Results:** The concentrations of tumor necrosis factor- α , interferon- γ , interleukin (IL)-10 and IL-12 in the cardiac blood sample of the infected mice were significantly higher than those in uninfected controls ($P<0.05$). The levels of transforming growth factor- β decreased in mice infected with *T. gondii* compared to those of the controls, the decrease was statistically significant ($P<0.05$). No significant difference was observed in levels of IL-4 between infected and healthy control groups ($P>0.05$). **Conclusions:** According to our findings, immune response into T helper type 1 was predominant during acute *T. gondii* infection. Further characterization and purification of *Toxoplasma* molecule(s) implicated in the regulation of cytokines could lead to the development of new drug prospects to control *Toxoplasma* infection.

1. Introduction

Toxoplasma gondii (*T. Gondii*) is an obligate intracellular parasite. It causes a variety of clinical syndromes that are usually asymptomatic in most immunocompetent individuals, but the infection is more severe in immunocompromised individuals and in cases of congenital toxoplasmosis[1]. Host protection to *T. gondii* infection results from a complex cell mediated immune response involving inflammatory cells, lymphocytes and macrophages, and cytokines[2]. Activated macrophages by interferon (IFN)- γ inhibit parasite replication through a number of potent microbicidal mechanisms, such as oxidative and non-oxidative mechanisms as well as the induction by IFN- γ of indoleamine 2,3-dioxygenase that degrades tryptophan, which is required for *T. gondii* replication. These mechanisms highlight the important role of IFN- γ to control the infection in immunocompetent hosts[3].

Briefly, the outcome of an infection with this parasite depends on a balance between pro-inflammatory

[interleukin (IL)-12, IFN- γ , tumor necrosis factor (TNF)- α] and anti-inflammatory (IL-10, lipoxin A4, IL-27) signals that suppress parasite proliferation and control the inflammatory response, respectively. Considering the lifelong presence of the parasite, hosts infected with *T. gondii* must develop a powerful immune response that has to be under strict control and such control should persistently be maintained in all infected tissues[4,5]. When endogenous IL-10 or transforming growth factor (TGF)- β was blocked in cell culture, it was observed that the parasite infection was under control only in the presence of IFN- γ [6].

Cell-mediated immune mechanisms play a major role in the control of *T. gondii* infection because the parasite is exclusively localized intracellularly. IFN- γ is the main cytokine involved with acute as well as with chronic resistance to *T. gondii* infection and recent studies have shown that the production and the response to IFN- γ must occur on both hematopoietic and nonhematopoietic cell lines in order to acquire an optimal protective host effect[7]. It has been demonstrated that these pro-inflammatory cytokines, especially IFN- γ , play a major role in the protection of the infected host, since they control tachyzoite growth[8].

Studies in the mouse model have provided important insights into how cellular immunity functions to control *T.*

*Corresponding author: Assoc. Prof. Isil Fidan, MD, Gazi University Faculty of Medicine, Department of Medical Microbiology 06500, Besevler, Ankara, Turkey.

Tel: +90 312 202 4626

Fax: +90 312 212 4647

E-mail: isilfidan@yahoo.com

gondii^[8–11]. Infection with the highly virulent RH strain led to widespread parasite dissemination and rapid death of mice^[9]. In this study, we aimed to determine the changes in the cytokine profiles acute toxoplasma infection in a mouse model of toxoplasmosis and thus, we could understand the immunological mechanisms formed in toxoplasma infection. Since a favourable outcome of toxoplasma infection depends on the balance between pro- and anti-inflammatory responses, we investigated the mouse serum levels of IL-12, IL-10, IL-4, TNF- α , IFN- γ and TGF- β .

2. Materials and methods

2.1. Animal model

Male mice ($n=8$) *Mus domesticus domesticus* at the age of 6–8 weeks and weighing between 18–21 g were selected for this study. These animals were maintained at Gazi University under conventional conditions. All experimental procedures on mice were consistent with International Guiding Principle for Biomedical Research Involving Animals and our research plan was approved by Local Ethics Committee for Experiments on Animals at Gazi University (No: 2006/06.025). The animals in infected group were inoculated with highly virulent *T. gondii* RH strain at a dose of 50.0 tachyzoites in 0.1 mL intraperitoneally. Eight animals were assigned in the healthy control group including same features. From each study group one mouse was sacrificed at 72th hours following the inoculation consecutively.

2.2. Cytokine levels

For cytokines assay, cardiac blood sample was collected. After centrifugation, the plasma was separated and stored at -80°C until they were analyzed. Levels of cytokines (TNF- α , IFN- γ , IL-12, IL-4, IL-6, TGF- β) were determined by specific enzyme-linked immunosorbent assay (ELISA) techniques according to the manufacturer's instructions (Biosource, California, USA). The concentration of cytokines was determined spectrophotometrically. The absorbance was read at 450 nm. We constructed a standard curve using cytokine standards. The cytokine concentrations for unknown samples were calculated according to the standard curve.

2.3. Statistical analysis

In control group and infected mouse group, cytokine levels were analysed using SPSS (13.0 version) with the Mann Whitney U test, and at $P<0.05$ was considered to be significant.

3. Results

In this study we observed that the concentrations of TNF- α , IFN- γ , IL-10 and IL-12 in infected mice were significantly

higher than those in uninfected controls ($P<0.05$). The levels of TGF- β decreased in mice infected with *T. gondii*, compared to those of the controls, the decrease was statistically significant ($P<0.05$) (Figure 1). There was no significant difference in levels of IL-4 between infected and healthy control groups ($P>0.05$) (The data was not shown).

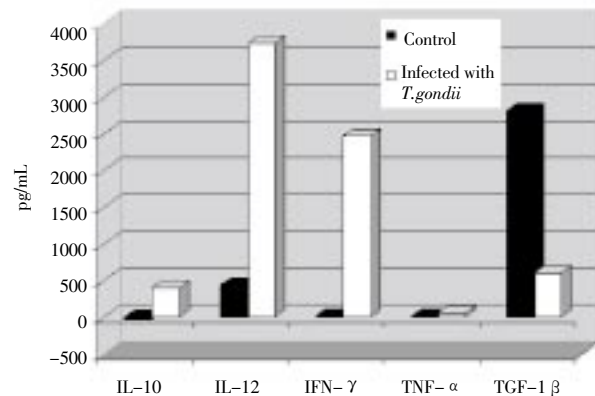


Figure 1. The cytokine levels of in infected and control groups.

4. Discussion

We found out that acute infection of mouse with *T. gondii* leads to the Th1 response, characterized by the production of cytokines such as TNF- α , IL-2, IL-12. While in mice infected with *T. gondii* the levels of IL-4, an indicator of Th2 response, did not differ significantly from those of controls, IL-10 levels were observed to increase significantly during *T. gondii* infection. In our study, regulatory cytokine TGF- β decreased in the infected group. Therefore, it was observed that the Th1 response was determined to be more dominant during acute *T. gondii* infection.

The immune response to *T. gondii* infection is complex, compartmentalized and it varies from individual to individual. This individual variation can be explained by the high level of heterogeneity in genetic background^[1]. Considering the complexity of the immunological events triggered during toxoplasmosis, the relevance of segregation of the immune response into types 1 and 2 still warrants further study. Cytokines have been shown to play an important role in the pathogenesis of toxoplasmosis. The induction of a type 1 inflammatory cytokine (IL-12, TNF- α and IFN- γ) response is a key event in the initiation of immunity to *T. gondii*^[12]. Moreover, this pro-inflammatory context may lead to a modulation of immune responses, either directed against parasite or unrelated antigens that develop in the host concomitantly with the infection. IFN- γ , the main cytokine responsible for immunological defense against *T. gondii*, is essential in all infected tissues. Since IFN- γ has been shown to be a potent inhibitor of Th2 cell proliferation, *T. gondii* infection may be expected to result in a preferential differentiation of T helper cells towards the Th1 type^[13].

The overproduction of type 1 cytokines, in particular IFN- γ , may lead to immune hyperactivity in susceptible hosts as observed in our model using *Mus domesticus* in

acute toxoplasmosis^[14]. An exacerbated and persistent inflammatory immune reaction mediated by IFN- γ stimuli would lead to a noxious cellular effect on host tissue, especially in the cerebral nervous system^[15]. Gene knock-out mice have established the importance of type 1 cytokines, such as IFN- γ , IL-12 and TNF- α , in the control of experimental toxoplasmosis. Absence of any one of these pro-inflammatory mediators results in increased mortality during infection as a result of uncontrolled tachyzoite growth^[8].

Nguyen *et al* detected that virulent strain RH induced in BALB/c mice is a type 1 cytokine pattern with T-cell-independent overproduction of IL-12 and IFN- γ . High levels of circulating IL-12 and IFN- γ were detected in the serum of mice infected with strain RH, although TNF- α levels remained low. Administration of antibody against IL-12 or IFN- γ significantly delayed time to death of mice infected with strain RH compared to controls^[10]. We observed that type 1 response was prominent in this study. High levels of circulating IL-12 and IFN- γ were detected in BALB/c mice infected with strain RH. At the same time, circulating TNF- α was remarkably increased in the sera of infected groups^[10,16]. Administration of anti-IL-12 or anti-IFN- γ antibodies in IL10-deficient mice resulted in delayed time for death. Moreover, SCID mice die from infection with low virulent strain ME49, which triggers prolonged overproduction of IL-12 and IFN- γ ^[17]. Mordue *et al* reported similar findings of acute toxoplasmosis leading to lethal overproduction of Th1 cytokines, including IL-12, IL-18 and IFN- γ , by using the high virulent strain RH^[14]. IL-10, which is produced by a variety of cell types (i.e., Th2 lymphocytes, macrophages, B cells, and mast cells), is now known to have multiple biologic activities, its major function appears to be inhibition of monokine (IL-1 β , TNF- α , and IL-12) synthesis by macrophages as well as down-regulation of IFN- γ production by CD4⁺ Th1 lymphocytes and NK cells. Paradoxically, infections with different intracellular pathogens, including *T. gondii*, induce both IL-10 and IFN- γ simultaneously^[2,3]. In our study, both IL-12 and IL-10 serum levels of infected mice were detected higher than healthy control group. IL-10 plays an important role in the balance between protective immunity and the development of immune pathology. IL-10 has been shown to act in the down-regulation of IFN- γ production in C57BL/6 mice following perioral infection with *T. gondii*, strain ME-49^[18]. It is well known that IL-10 also inhibits the production of IL-12, TNF- α and IL-6 and that *T. gondii* infection is dominated by a strong type 1 response, characterized by high systemic levels of IL-12 and IFN- γ ^[19]. To examine the function of IL-10 synthesis during early infection with the intracellular protozoan *T. gondii*, IL-10 knockout mice were inoculated with an avirulent parasite strain (ME-49) levels of IL-12 and IFN- γ in sera of infected IL-10 deficient animals were four to sixfold higher than those in sera from control mice. Early IL-10 production may serve an additional and perhaps more important function; protection of the host against the detrimental effects of an excessive cellular immune response elicited during acute infection^[8,20]. In toxoplasmosis, the *in vivo* administration of an anti-IL-10

monoclonal antibody to SCID mice delays the death of the animals following *Toxoplasma* infection^[21]. *In vivo* studies have also shown that resistance to acute toxoplasmosis can be abrogated by treatment with neutralizing Abs against IFN- γ , TNF- α or IL-12^[20]. Aldebert *et al* demonstrated that infection by live parasites (RH strain) triggers secretion of IL-12, but low level of IL-10 in human monocyte cell line^[22]. Early stimulation of macrophages also plays an important role in directing cell mediated immunity since IL-12 promotes Th1-type acquired immunity, which is essential to control *Toxoplasma* infection. The balance between IL-12 and IL-10 is thus essential to control *Toxoplasma* infection^[23].

IL-4 is secreted by type-2 lymphocyte. IL-4 alone does not appear to influence the intracellular growth of *Toxoplasma in vitro*^[24]. Nevertheless, in mice models, endogenous IL-4 plays a significant role in resistance to *T. gondii*^[25]. However, Calabrese *et al* did not detect any significant levels of IL-4 in serum between infected mice with low virulent strain ME-49 and uninfected control group^[11]. According to our findings, there was no difference about IL-4 serum levels between infected and non-infected mice group.

TGF-1 β is anti-inflammatory cytokine and produced by virtually all types of cells and plays an important role in immunoregulatory processes. TGF-1 β is considered to be an antagonist of TNF- α , IFN- γ , TNF-1 β and IL-2. The anti-inflammatory action of this cytokine makes it possible to control the development of immunopathological phenomena related to a type 1 immune response in the intestines or the brain^[2]. The inhibitory effect of TGF-1 β on toxoplasmatatic activity is mediated via inhibition of TNF- α demonstrated an essential role of TNF- α in the IFN- γ -induced generation of these activities of murine macrophages. For the induction of these activities, the IFN- γ induced production of TNF- α during the initial phase of macrophage activation, prior to *T. gondii* infection, is essential^[26]. In this study, we determined lower level of TGF-1 β in serum of infected mice than control group.

Our findings suggest that depending on host factors, virulence of *T. gondii* strains, duration of infection, the cytokine profiles changed, protective or regulatory cytokine response were dominant. Further characterization and purification of *Toxoplasma* molecule(s) implicated in the regulation of cytokines could lead to the development of new drug prospects to control *Toxoplasma* infection.

Conflict of interest statement

We declare that we have no conflict of interest.

References

- [1] Remington JS, McLeod R, Thulliez P, Desmonts G. Toxoplasmosis. In: Remington JS, Klein OJ, eds. *Infection diseases of the fetus and newborn infant*. Philadelphia: W. B. Saunders; 2001.
- [2] Filisetti D, Candolfi E. Immune response to *Toxoplasma gondii*.

- Ann Ist Super Sanità* 2004; **40**: 71–80.
- [3] Gaddi PJ, Yap GS. Cytokine regulation of immunopathology in toxoplasmosis. *Immun Cell Biol* 2007; **85**: 155–9.
- [4] Bhopale GM. Pathogenesis of toxoplasmosis. *Comp Immunol Microbiol Infect Dis* 2003; **26**: 213–22.
- [5] Aliberti J. Host persistence: exploitation of anti-inflammatory pathways by *Toxoplasma gondii*. *Nat Rev Immunol* 2005; **5**: 162–70.
- [6] Barbosa BF, Silva DAO, Costa IN, Mineo JR, Ferro EAV. BeWo trophoblast cell susceptibility to *Toxoplasma gondii* is increased by interferon- γ , interleukin-10 and transforming growth factor- β 1. *Clin Exp Immunol* 2008; **151**: 536–45.
- [7] Yano A, Mun HS, Chin M, Norose K, Hata K, Kobayashi M, et al. Roles of IFN- γ on stage conversion of an obligate intracellular protozoan parasite, *Toxoplasma gondii*. *Int Rev Immunol* 2002; **21**: 405–21.
- [8] Gazzinelli RT, Wysocka M, Hieny S, Schariton-Kersten T, Cheever A, Kuhn R, et al. In the absence of endogenous IL-10, mice acutely infected with *Toxoplasma gondii* succumb to a lethal immune response dependent on CD4⁺ T cells and accompanied by overproduction of IL-12, IFN- γ and TNF- α . *J Immunol* 1996; **157**: 798–805.
- [9] Gavrilescu LC, Denkers EY. IFN- γ overproduction and high level apoptosis are associated with high but not low virulence *Toxoplasma gondii* infection. *J Immunol* 2001; **167**: 902–9.
- [10] Nguyen TD, Bigaignon G, Markine-Goriaynoff D, Uyttenhove C, Van Snick J, Coutelier JP, et al. Virulent *Toxoplasma gondii* strain RH promotes T-cell-independent overproduction of proinflammatory cytokines IL12 and γ -interferon. *J Med Microbiol* 2003; **52**: 869–76.
- [11] Calabrese KS, Tedesco RC, Zaverucha do Valle T, Barbosa HS. Serum and aqueous humour cytokine response and histopathological alterations during ocular *Toxoplasma gondii* infection in C57BL/6 mice. *Micron* 2008; **39**: 1335–41.
- [12] Jones LA, Roberts CW. Ocular toxoplasmosis: in the storm of the eye. *Parasite Immunol* 2006; **28**: 635–42.
- [13] Gajewski TF, Fitch FW. Anti-proliferative effect of IFN- γ in immune regulation. I. IFN- γ inhibits the proliferation of Th2 but not Th1 murine helper T lymphocyte clones. *J Immunol* 1988; **140**: 4245–52.
- [14] Mordue DG, Monroy F, La Regina M, Dinarello CA, Sibley LD. Acute toxoplasmosis leads to lethal overproduction of Th1 cytokines. *J Immunol* 2001; **167**: 4574–84.
- [15] Rozenfeld C, Martinez R, Seabra S, Sant'anna C, Goncalves JG, Bozza M. *Toxoplasma gondii* prevents neuron degeneration by interferon- γ -activated microglia in a mechanism involving inhibition of inducible nitric oxide synthase and transforming growth factor- β 1 production by infected microglia. *Am J Pathol* 2005; **167**: 1021–31.
- [16] Yap G, Pesin M, Sher A. Cutting edge: IL-12 is required for the maintenance of IFN- γ production in T cells mediating chronic resistance to the intracellular pathogen, *Toxoplasma gondii*. *J Immunol* 2000; **165**: 628–31.
- [17] Walker W, Roberts CW, Ferguson DJP, Jebbari H, Alexander J. Innate immunity to *Toxoplasma gondii* is influenced by gender and is associated with differences in interleukin-12 and gamma interferon production. *Infect Immun* 1997; **65**: 1119–21.
- [18] Suzuki Y, Sher A, Yap G, Park D, Neyer LE, Liesenfeld O, et al. IL-10 is required for prevention of necrosis in the small intestine and mortality in both genetically resistant BALB/c and susceptible C57BL/6 mice following peroral infection with *Toxoplasma gondii*. *J Immunol* 2000; **164**: 5375–82.
- [19] Wilson EH, Wille-Reece U, Dzierszinski F, Hunter CA. A critical role for IL-10 in limiting inflammation during toxoplasmic encephalitis. *J Neuroimmunol* 2005; **165**: 63–74.
- [20] Khan IA, Matsuura T, Kasper LH. Interleukin-12 enhances murine survival against acute toxoplasmosis. *Infect Immun* 1994; **62**: 1639–42.
- [21] Neyer LE, Grunig G, Fort M. Role of interleukin-10 in regulation of T-cell-dependent and T-cell-independent mechanisms of resistance to *Toxoplasma gondii*. *Infect Immun* 1997; **65**: 1675–82.
- [22] Aldebert D, Durand F, Mercier C, Brenier-Pinchart MP, Cesbron-Delauw MF, Pelloux H. *Toxoplasma gondii* triggers secretion of interleukin-12 but low level of interleukin-10 from the THP-1 human monocytic cell line. *Cytokine* 2007; **37**: 206–11.
- [23] Denkers EY, Gazzinelli RT. Regulation and function of T-cell-mediated immunity during *Toxoplasma gondii* infection. *Clin Microbiol Rev* 1998; **11**: 569–88.
- [24] Appelberg R, Orme I, Pinto de Souza M, Silva M. *In vitro* effects of interleukin-4 on interferon-gamma induced macrophage activation. *Immunol* 1992; **76**: 553–9.
- [25] Villard O, Candolfi E, Despringre J, et al. Protective effect of low doses of an anti-IL-4 monoclonal antibody in a murine model of acute toxoplasmosis. *Parasite Immunol* 1995; **17**: 233–6.
- [26] Langermans JA, Nibbering PH, Van Vuren-Van Der Hulst ME, Van Furth R. Transforming growth factor-beta suppresses interferon-gamma induced toxoplasmastatic activity in murine macrophages by inhibition of tumour necrosis factor-alpha production. *Parasite Immunol* 2001; **23**: 169–75.